**DOCKET NO.:** .: UBCV-0006/ 01-010 **PATENT** 

**Application No.:** 10/039,760

Notice of Allowance Dated: January 10, 2007

## Amendments to the Specification:

Please amend the paragraph beginning on page 15, line 22, to page 16, line 9, as follows:

Generally, CCS is produced by culturing EHEC bacteria in a suitable medium, under conditions that favor type III antigen secretion. Suitable media and conditions for culturing EHEC bacteria are known in the art and described in e.g., U.S. Patent Nos. 6,136,554 and 6,165,743 (incorporated herein by reference in their entireties), as well as in Li et al., *Infec.* Immun. (2000) 68:5090-5095; Fey et al., Emerg. Infect. Dis. (2000) Volume 6. A particularly preferable method of obtaining CCS is by first growing organisms in Luria-Bertani (LB) medium for a period of about 8 to 48 hours, preferably about 12 to 24 hours, and diluting this culture about 1:5 to 1:50, preferably 1:5 to 1:25, more preferably about 1:10, into M-9 minimal medium supplemented with 20-100 mM NaHCO<sub>2</sub> NaHCO<sub>3</sub>, preferably 30-50 mM, most preferably about 44 mM NaHCO2 NaHCO3, 4-20 mM MgSO4, preferably 5-10 mM and most preferably about 8 mM MgSO<sub>4</sub>, 0.1 to 1.5% glucose, preferable 0.2 to 1% most preferably 0.4% glucose and 0.05 to 0.5% Casamino Acids, preferably 0.07 to 0.2%, most preferably about 0.1% Casamino Acids. Cultures are generally maintained at about 37 degrees C in 2-10% CO<sub>2</sub>, preferably about 5% CO<sub>2</sub>, to an optical density of about 600nm of 0.7 to 0.8. Whole cells are then removed by centrifugation and the supernatant can be concentrated, e.g. 10-1000 fold or more, such as 100-fold, using dialysis, ultrafiltration and the like. Total protein is easily determined using methods well known in the art.

Please amend the paragraph on page 24, lines 21-28, as follows:

Wild type EHEC O157:H7 were grown under conditions to maximize the synthesis of CCS proteins (Li et al., *Infect. Immun*. (2000) <u>68</u>:5090). Briefly, and overnight standing culture of EHEC O157:H7 was grown in Luria-Bertani (LB) medium overnight at 37°C (±5% CO<sub>2</sub>). The culture was diluted 1:10 in M-9 minimal medium supplemented with 0.1% Casamino Acids, 0.4% glucose, 8 mM MgSO<sub>4</sub> and 44 mM NaHCO<sub>2</sub> NaHCO<sub>3</sub>. Cultures were grown standing at 37°C 5% CO<sub>2</sub> to an optical density at 600 nm of 0.7 to 0.8 (6-8 h). Bacteria were removed by centrifugation at 8000 rpm for 20 min at 4°C. The supernatant was concentrated 100 fold by ultrafiltration and total protein was determined by the bicinchoninic acid protein assay method.